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PHENTOLAMINE I.V. CAUSES REDUCTION IN THE SIZE OF RECEPTIVE FIELDS OF LOW THRESHOLD SPINAL NEURONS

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Abstract : Intravenous (i.v.) phentolamine sometimes alleviates sensory abnormalities in patients with sympathetically-maintained pain. Alternation in activity of the central neurons may underlie the phenomenon. We hypothesized changes in the receptive fields (RF) of spinal neurons occur after i.v.phentolamine in physiological conditions as well as in the painful pathological conditions. To test this hypothesis in part, we investigated the effect of i.v. phentolamine on the size of RF of low threshold (LT) spinal neurons in intact rats under halothane anesthesia supplemented with ketamine. After confirmation of the phentolamine-induced reduction in the size of RF in LT neurons, we studied the degree of phentolamine-induced maximal reduction in the size of RF, reversibility of the change, and effect of yohimbine on the reduction in 12 rats. Those rats were divided into two groups. Following recovery of the RF size to their baseline levels on the first phentolamine (1mg/kg) i.v., the same dose of i.v. phentolamine was repeated 20 min after i.v. administration of either saline (saline group: n=5) or yohimbine (0.5mg/kg, yohimbine group: n=7). Results showed that i.v. phentolamine significantly reduced the size of RF at the first i.v. phentolamine, where the means of maximum reductions were 62.5% and 66.6% (mean % vs control) in the saline and yohimbine groups, respectively. The phentolamine-induced maximum reduction in RF size occurred 10 to 30 min after phentolamine administration, where the RF recovered to their baseline levels 10 to 30 min after the maximum reduction in most of the cases. The second phentolamine-induced reduction in RF size was 10.3% after yohimbine pretreatment, whereas it was 66.8% after saline treatment. We provided here the first evidence that i.v. phentolamine produced a yohimbine-reversible reduction in LT neuron RF size. Our results could indicate that sympathetic blockade with i.v. phentolamine results in inhibitory modulation of spinal neuronal activity.

Key words : phentolamine, receptive field, spinal neuron, sympathetically-maintained pain

Introduction

Intravenous (i.v.) phentolamine, that acts mainly on the peripheral alpha-1 and -2 adrenergic receptors and blocks the sympathetic nerve, can sometimes reduce pain intensity in patients with chronic pain[1-5]. Pain reduced by sympathetic block is called sympathetically-maintained pain (SMP)[6,7], where a single dose of i.v. phentolamine has been used to diagnose SMP[4,8]. Sympathetic blockade sometimes reduces the size of area of sensory abnormalities, such as dynamic mechanical allodynia and tactile hypoesthesia in patients with SMP[2,3,5], which seemingly suggest that sympathetic blockade may result in modulation of pain processing in the central nervous system. Expansion of receptive fields (RF) of dorsal horn neurons may produce hyperesthesia and pain in neuropathic pain[8], which is sometimes accompanied by SMP. Previous reports provide evidence that expansion of RFs of low threshold (LT) as well as wide dynamic range (WDR) neurons in the dorsal spinal cord did occur in animals with neuropathic pain models[9-11]. Although those facts were shown in the painful pathological conditions, we have postulated that common central effects of the sympathetic nerve blockade on the activity of spinal neurons both in the pathological and physiological conditions exist and hypothesized that reduction of RF of LT neurons occur after peripheral sympathetic blockade in non-pathological conditions. To test this hypothesis in part, we investigated the effect of i.v. phentolamine on the size of RF of spinal neurons in intact rats. We also investigated whether i.v.yohimbine, which can block spinal noradrenergic descending system[12], could affect the possible phentolamine-induced changes of the neurons.

Materials and methods

Animals and preparations: With approval of the Institutional Animal Care and Use Committee of Yale University, the extracellular activity of single spinal dorsal horn LT neurons was recorded in intact male Sprague-Dawley rats (340-380 g;

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Harlan Sprague-Dawley, Inc., Indianapolis, IN). Anesthesia for surgical preparation of the animals was induced with halothane 2-4% in 100% oxygen. The arterial line was placed in the right carotid artery to monitor arterial pressure. The i.v. catheter was inserted to the left extra-jugular vein. After establishment of the i.v. line, the animals were given ketamine 0.2-0.5 mg/kg intravenously before laminectomy and paralyzed with repeated doses of pancuronium(0.2mg). Using a respirator, the lungs were ventilated through the tracheal tube after tracheotomy. Anesthesia was maintained with 0.5-1.5% halothane in 100% oxygen. Decrease of the arterial pressure was treated with decreased halothane concentration or incremental dose of saline administration if hypovolemia was suspected. Systolic arterial pressure was maintained within a normal range of 70 to 120mmHg throughout the experiments. Data in animals experienced mean arterial pressure less than 50 mmHg were eliminated from our results to avoid effect of hypotension on LT-RF[13]. End-tidal PCO_2 (25-35 mmHg) and rectal temperature (36-37℃) were monitored and maintained throughout experiments.

Extracellular recordings and determination of the size of receptive field: We made extracellular recordings and determined the RF of LT spinal neurons to light touch (von Frey filament) with mapping and marked on the shaved skin according to the previous reports[14]. Briefly, the lumbar spinal cord (L2-5) was exposed by a laminectomy. For the recording of single unit activity, a tungsten microelectrode (impedance, 10 MΩ; FHC Inc., Brunswick, ME) was inserted, and a single LT neuron was identified by the characteristic response profile as neurons that were excited maximally by innocuous tactile stimulation with a brush. Edge of the RF area was determined as those points where light touch with a von Frey hair elicited a response of 50% of the time. After determination of stable baseline RF size, RFs were plotted on the surface of the skin 5 (in the pilot study) and every 10 min after each treatment. LT neuronal recordings were converted to digital signals and stored (CED 1401 Plus; Cambridge Electronic Design Ltd., Cambridge, UK). All data were subsequently analyzed with Spike 2 software program (Cambridge Electrical Design Ltd.) For standardization of analysis, raw data were converted to percent control.

Study protocols: 1) Pilot study : In order to test occurance of phentolamine-induced changes in the size of RF, we examined 10 LT neurons. 2) Reversibility of the phentolamine-induced changes in the RF size (Fig. 1A,B) : Saline was administered intravenously immediately after recovery of the RF size to the baseline levels on the first phentolamine (1mg/kg, dissolved in saline) i.v. The same dose of i.v. phentolamine was repeated 20 min after administration of saline (saline group: n=5). 3) Effect of yohimbine treatment in the phentolamine- induced changes in the RF size (Fig. 1A,B) : Yohimbine (0.5mg/kg) was administered immediately after recovery of the RF size to their baseline levels on the first phentolamine (1mg/kg, dissolved in saline) i.v., the same dose of i.v. phentolamine was repeated 20 min after i.v.administration of yohimbine (yohimbine group: n=7).

Data Analysis: Significance of phentolamine-induced maximum changes of the size of RF was tested in each experiment group. Those maximum changes were compared among groups. Statistical analysis of data was carried out using a non-parametric Wilcoxon signed-ranks test for paired data, Mann-Whitney U test for unpaired data, Friedmann test for comparisons of groups for paired data and Kruskal-Wallis test for comparison of those of unpaired data. P values less than 0.05 were accepted as significant. Data were expressed mean +/- S.D. when indicated.

Results

General observations: The phentolamine-induced maximum reduction in RF size occurred 10 to 30 min after phentolamine administration, where the RF recovered to their baseline levels 10 to 30 min after the maximum reduction in most of the cases. Both phentolamine and yohimbine i.v. caused a transient decrease of arterial pressure. However, the arterial pressure recovered spontaneously to baseline levels within 5 min in most of the cases.

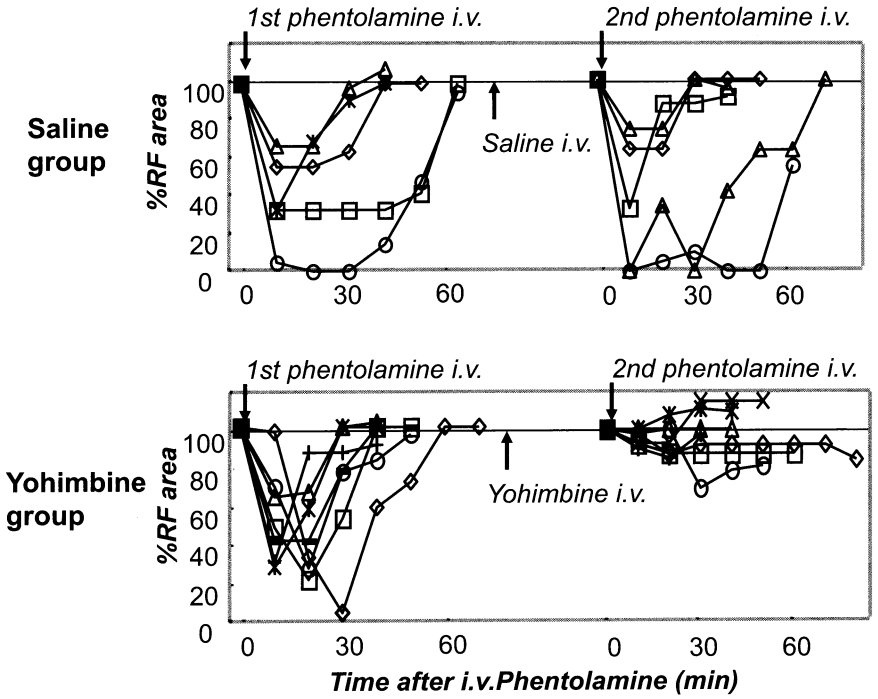
Pilot study: Phentolamine (1mg/kg) i.v. did induce a significant reduction in the size of RF in 10 LT neurons. The mean % reduction of the receptive field area was 61.5%.

Reversibility of the phentolamine-induced changes in the RF size in saline group: I.v. phentolamine. significantly reduced the size of RF after the first as well as the second administration (Fig. 1A,B). Phentolamine-induced mean maximum reduction in RF size was 62.5% for the first, and 66.6% for the second i.v. phentolamine, respectively (Fig. 1B).

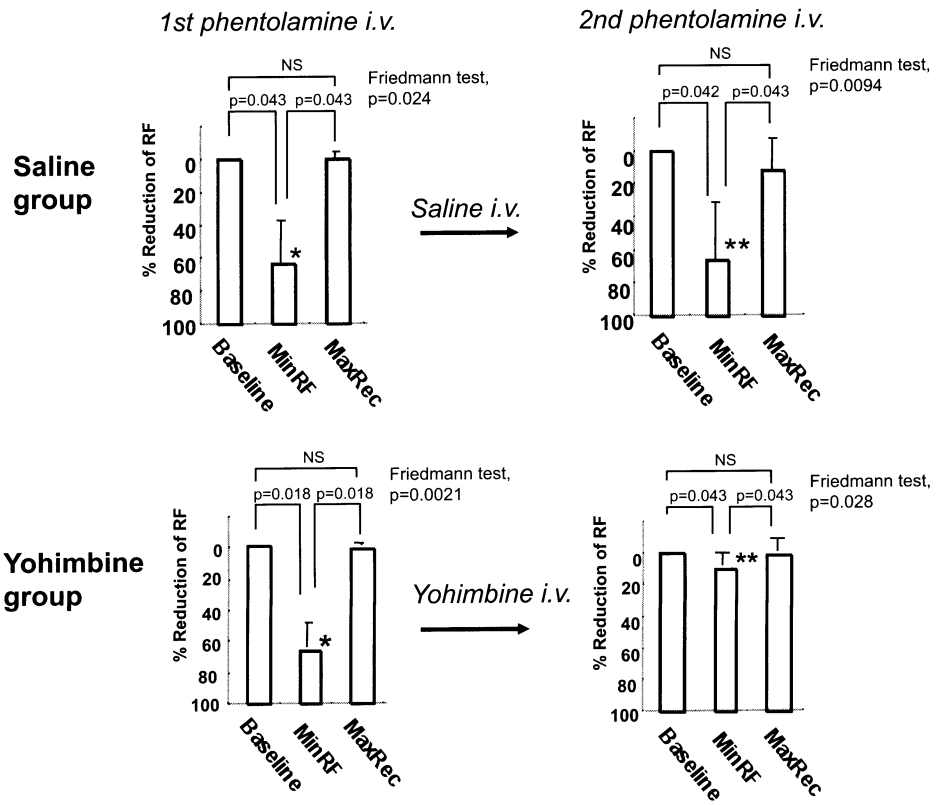
Effect of yohimbine treatment on the phentolamine-induced changes of the RF size in yohimbine group: I.v. phentolamine significantly reduced the size of RF after the first administration. However, the reduction after the second administration following yohimbine pretreatment was much less than that of the first administration (Figs 1A,B). Phentolamine-induced mean maximum reduction in RF size was 66.8% and 10.3% for the the first and second i.v. phentolamine. after yohimbine treatment, respectively (Fig. 1B).

Comparison of % RF areas at maximum reduction among groups: %RF area at maximum reduction after the second i.v. phentolamine following yohimbine treatment was significantly larger than any other of those areas, which indicates phentolamine-induced reduction in RF size was significantly inhibited with yohimbine pretreatment (Fig. 2).

RF size after yohimbine i.v.: Yohimbine itself administered immediately after recovery of the RF size to their baseline levels on the first i.v. phentolamine did not alter the sizes of RF during 15 min after its administration when compared the size just before i.v. yohimbine (Fig. 3).



Figures 1. A



Figures 1. B

Figures 1. A, B: Changes of %RF areas after first and second i.v. phentolamine in saline and yohimbine groups. In the saline group, phentolamine-induced mean maximum reduction in RF size was 62.5% for the first, and 66.6% for the second i.v. phentolamine, respectively. However, the reduction after the second administration following yohimbine pretreatment was much less than that after the first administration. In the yohimbine group, phentolamine-induced mean maximum reduction in RF size was 66.8% for the first, and 10.3% for the second i.v. phentolamine, respectively.

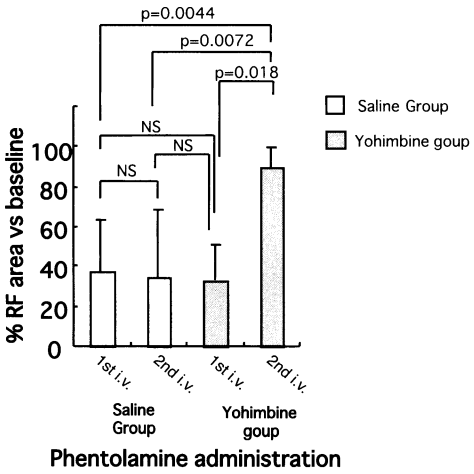


Figure 2. Phentolamine-induced reduction in RF size was significantly inhibited with yohimbine pretreatment.

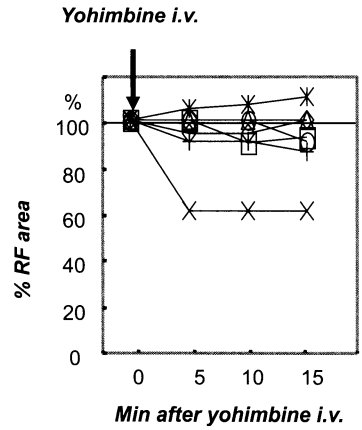


Figure 3. Yohimbine itself administered immediately after recovery of the RF size to their baseline levels on the first i.v. phentolamine did not alter the sizes of RF.

Discussions

In the present study, we provided first evidence that i.v. phentolamine produced a yohimbine-reversible reduction in LT neuron RF size in intact rats under halothane anesthesia supplemented with ketamine. Our results seemingly suggest that sympathetic blockade with i.v. phentolamine causes inhibitory modulation of spinal neuronal activity in non-pathological conditions.

Although spinal drug actions could exist, direct phentolamine action on the spinal cord seems unlikely to explain the present results. Phentolamine blocks alpha-1 and-2 adrenoreceptors [4,11,15], where i.v. phentolamine has little effect on the central neuronal activity because it does not readily penetrate the blood-brain barrier[11]. When administered intravenously, phentolamine acts mainly on the peripheral system and blocks activities of efferent sympathetic nerves[11] . If phentolamine is given intrathecally, it can inhibit activity of the descending noradrenergic system[15], which should result in disinhibition of spinal neuronal activity and expansion of LT neuron RF size. On the other hand, yohimbine alpha-2 adrenoreceptor antagonist, when given intravenously, inhibits both the peripheral and central alpha-2 adrenergic systems, the latter of which includes noradrenergic descending inhibitory system[12]. Assuming that i.v. phentolamine has direct spinal drug action, phentolamine given after yohimbine is expected to work synergistically with yohimbine to counteract reduction of spinal neuronal activity and result in expansion of the RF in conditions where noradrenergic descending inhibitory system is involved. Phentolamine, however, produced reduction in LT neuron RF size in the present study. Although spinal drug actions other than mentioned above could exist, direct spinal drug action of phentolamine seems not to elucidate the results of present study as far as alpha-2 adrenoreceptor mechanisms are considered.

It is noteworthy that the maximum reduction in LT neuron RF size occurred 10 to 30 min after i.v. phentolamine, while maximum decrease in the arterial pressure occurred within 5 min after i.v. phentolamine and the arterial pressure recovered spontaneously after a few minutes in the most of our cases. One possible explanation for the delayed response of LT neuron RF size to i.v. phentolamine is that the reflex-released noradrenaline and adrenaline after i.v. phentolamine act on the spinal cord and reduce in LT neuron RF size through spinal noradrenergic inhibitory mechanism. However, the highest values of plasma noradrenaline and adrenaline concentrations occur earlier from 2 to 6 min after i.v. phentolamine in human[16]. If elevation of those catecholamines occurred in this timing in our rats, it seems not likely to elucidate the delayed response of LT neuron RF size with such reflex-released catecholamines.

If we postulate that i.v. phentolamine indirectly enhances the descending noradrenergic system, it is possible to elucidate results of our present study. The yohimbine-reversibility of phentolamine-induced reduction of RF-LT neurons found in the present study seems to suggest that alpha-2 adrenergic descending inhibitory system is involved in the mechanism[17]. In the present study, we did not directly investigate the feedback mechanism; however, such mechanism is likely to occur if we consider the close relationships among the noradrenergic descending inhibitory pathways and peripheral and central sympathetic pathways[18]. If the central sympathetic outflow system links with the descending noradrenergic inhibitory system, it could explain both the transient reduction in LT neuron RF size that occurred after certain latent time.

In this present study, we demonstrated that i.v. phentolamine reduced LT neuron RF size. However, our results are limited to animals with non-pathological conditions and might not be applicable to patients and animals with SMP. Further study

could show whether such reduction in WDR neuron RF size and LT neuron RF size could occur in neuropathic pain models.

In conclusion, we provided here the first evidence that i.v. phentolamine produced a yohimbine-reversible reduction in LT neuron RF size in intact rats. Our present study indicates that peripheral sympathetic blockade can result in modulation of spinal neuronal activity even in the non-pathological conditions.

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